

Claims

1. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

2. A device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, in a sequence sufficiently duplicative of the sequence of

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COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

3. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CXXXXLXVXFDXGWXXWXXXPXGXXAXYCXGXCPXXXXXXXNHAXX				
60	70	80	90	100
QXXVXXNXXXXPXXCCXPXXXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX				

wherein each X independently represents an amino acid.

4. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
LXVXFDXGWXXWXXXPXGXXAXYCXGXCPXXXXXXXNHAXX				
60	70	80	90	100
QXXVXXNXXXXPXXCCXPXXXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX				

wherein each X independently represents an amino acid.

5. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECFPFLADHLNSTNHAIV				
RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI				
KE F E K I DN L N S Q ITK F P TL				
Q A S K				
60	70	80	90	100
QTLVNSVNPGKIPKACCVPTLSAISMLYLDENENVVLKNYQDMVVEGCGCR				
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H				
RF T S K DPV V Y N S H RN RS				
N S K P E				

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

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6. The device of claim 1 or 2 wherein the sequence comprises:

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      10      20      30      40      50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECFFPLADHLNSTNHAIV
  K S S L  QE VIS E FD Y  E A AY MPESMKAS  VI
  F E K I  DN   L   N S   Q  ITK F P   TL
      A   S   K
      60      70      80      90      100
QTLVNSVNP G K I P K A C C V P T E L S A I S M L Y L D E N E N V L K N Y Q D M V V E G C G C R
SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
  RF  T   S   K DPV V  Y N S   H RN  RS
  N   S               K       P   E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

7. The device of claim 1 or 2 wherein the sequence comprises:

```
      1      10      20      30      40
Vg1  CKKRHLVVEFK-DVGWQNWVIA PQGYMAN YCYGEC PYPLTE
      50      60      70
      ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
      80      90      100
      ISMLFYDNNNDNVVLRHYENMAVDECGCR
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8. The device of claim 1 or 2 wherein the sequence comprises:

```
      1      10      20      30      40
DPP  CRRHSLYVDFS-DVGWDDWIVAPLG YDAY YCHGKCPFLAD
      50      60      70
      HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
      80      90      100
      VAMLYLNDQSTVV LKN YQEMTVVGCGCR
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9. The device of claim 1 or 2 wherein the sequence comprises:

```
      1      10      20      30      40
OP1  LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFFPLNS
      50      60      70
      YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
      80      90      100
      ISVLYFDDSSNVILKKYRNMVVRACGCH
```

10. The device of claim 1 or 2 wherein the sequence comprises:

-5  
HQRQA

	1	10	20	30	40
OP1	CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFP	LNS			
		50	60	70	
	YMNATN--H-AIVQTLVHF	INPET-VPKPCCAPTQLNA			
	80	90	100		
	ISVLYFDDSSNVILKKYRNMVVRACGCH				

11. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-2a	CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGEC	PFPLAD			
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPT	ELSA			
	80	90	100		
	ISMLYLDENEKVVVLKNYQDMVVEGCGCR				

12. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-2b	CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDC	PFPLAD			
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPT	ELSA			
	80	90	100		
	ISMLYLDEYDKVVVLKNYQEMVVEGCGCR				

13. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-3	CARRYLKVDFA-DIGWSEWIIISP	KSFDAYYCSGACQFPMPK			
		50	60	70	
	SLKPSN--H-ATIQSIVRAVG	VVPGIPEPCCVPEKMSS			
	80	90	100		
	LSILFFDENKNVVLKVYPNMTVESCACR				

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14. The device of claim 1 or 2 wherein the sequence comprises:

COP1      1            10            20            30            40  
                  LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD  
                                  50            60            70  
                  HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA  
                                  80            90            100  
                  ISMLYLDENSTVVLKNYQEMTVVGCGCR

15. The device of claim 1 or 2 wherein the sequence comprises:

COP3      1            10            20            30            40  
                  LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD  
                                  50            60            70  
                  HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA  
                                  80            90            100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

16. The device of claim 1 or 2 wherein the sequence comprises:

COP4      1            10            20            30            40  
                  LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD  
                                  50            60            70  
                  HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA  
                                  80            90            100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

17. The device of claim 1 or 2 wherein the sequence comprises:

COP5      1            10            20            30            40  
                  LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPPFLAD  
                                  50            60            70  
                  HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTLSA  
                                  80            90            100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

18. The device of claim 1 or 2 wherein the sequence comprises:

COP7      1            10            20            30            40  
                  LYVDFS-DVGWDDWIVAPPGYHAFYCHGECPPFLAD  
                                  50            60            70  
                  HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTLSA  
                                  80            90            100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

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19. The device of claim 1 or 2 wherein the sequence comprises:

10  
PKHHSQRARKKNKN  
1 10 20 30 40  
COP16 CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPPFLAD  
50 60 70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

20. The device of claim 1 or 2 wherein the osteogenics protein comprises a pair of separate polypeptide chains.

21. Osteogenic protein, produced by expression of recombinant DNA in a host cell, capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

22. A protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long in a sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

23. <sup>22</sup> The osteogenic protein of claim <sup>22</sup> having an ~~apparent~~ molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel.

<sup>3</sup>  
24. The osteogenic protein of claim <sup>2</sup>23 further characterized by being glycosylated.

<sup>4</sup>  
25. The osteogenic protein of claim <sup>125</sup>21 having an apparent molecular weight of about 27 kD as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

<sup>5</sup>  
26. The protein of claim <sup>21 1 4</sup>22 or 25 further characterized by being unglycosylated.

27. The protein of claim 21 or 22 comprising a pair of separate polypeptide chains.

<sup>6</sup>  
28. The protein of claim ~~21 or 22~~ comprising the amino acid sequences:

10 20 30 40 50  
CXXXLXVXFDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX  
60 70 80 90 100  
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

wherein each X independently represents an amino acid.

<sup>7</sup>  
29. The protein of claim ~~21 or 22~~ comprising the amino acid sequences:

10 20 30 40 50  
LXVXFDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX  
60 70 80 90 100  
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

wherein each X independently represents an amino acid.

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30. The protein of claim 21 or 22 comprising the amino acid sequences:

	10	20	30	40	50
CKRHPLYVDFRDVGW	NDWIVAPPGYHAFY	CHGECPPFLADHLN	STNHAIV		
RRRS K S S L	QE VIS E FD Y	E A AY MPESMKAS	VI		
KE F E K I	DN L N S	Q ITK F P	TL		
Q A S K					
60	70	80	90	100	
QTLVNSVNP	GKIPKACCVPT	ELSAISMLYLD	ENENVVLK	NYQDMVVEG	CGCR
SI HAI SEQV	EP A EQMNSL	AI FFNDQDK	I RK EE T	DA H H	
RF T S	K DPV V	Y N S	H RN RS		
N S		K	P	E	

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

31. The protein of claim 21 or 22 comprising the amino acid sequences:

	10	20	30	40	50
LYVDFRDVGW	NDWIVAPPGYHAFY	CHGECPPFLADHLN	STNHAIV		
K S S L	QE VIS E FD Y	E A AY MPESMKAS	VI		
F E K I	DN L N S	Q ITK F P	TL		
A S K					
60	70	80	90	100	
QTLVNSVNP	GKIPKACCVPT	ELSAISMLYLD	ENENVVLK	NYQDMVVEG	CGCR
SI HAI SEQV	EP A EQMNSL	AI FFNDQDK	I RK EE T	DA H H	
RF T S	K DPV V	Y N S	H RN RS		
N S		K	P	E	

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

32. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
Vg1	CKKRHL	YVEFK-DVGW	QNWVIA	PPGYMAN	YCYGEC
		50	60	70	
	ILNGSN	--H-AILQ	TLVHST	EPED-IPL	PCCVPT
	80	90	100		
	ISMLFY	DNNDNV	VLRLHY	ENMAV	DECGCR

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33. The protein of claim 21 or 22 comprising the amino acid sequences:

DPP 1 10 20 30 40  
CRRHSLYVDFS-DVGWDDWIVAPLGDAYYCHGKCPFFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS  
80 90 100  
VAMLYLNDQSTVVLKNYQEMTVVGCGCR

34. The protein of claim ~~21~~ or ~~22~~ comprising the amino acid sequence:

OP1 1 10 20 30 40  
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

35. The protein of claim ~~21~~ or ~~22~~ comprising the amino acid sequences:

OP1 1 10 20 30 40  
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

36. The protein of claim 21 or 22 comprising the amino acid sequences:

CMP-2a 1 10 20 30 40  
CKRHPLYVDFS-DVGWDDWIVAPPGYHAFYCHGECPPFLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTQLSA  
80 90 100  
ISMLYLDENEKVVVLKNYQDMVVEGCGCR

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37. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
CBMP-2b	CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD				
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA				
	80	90	100		
	ISMLYLDEYDKVVLKNYQEMVVEGCGCR				

38. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
CBMP-3	CARRYLKVDFA-DIGWSEWIIISPKSFDAYYCSGACQFPMPK				
		50	60	70	
	SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS				
	80	90	100		
	LSILFFDENKNVVLKVYPNMTVESACR				

39. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
COP1	LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD				
		50	60	70	
	HFNSTN--H-AVVQTLVNNMNPCK-VPKPCCVPTELSA				
	80	90	100		
	ISMLYLDENSTVVLKNYQEMTVVGCGCR				

40. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
COP3	LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD				
		50	60	70	
	HFNSTN--H-AVVQTLVNNMNPCK-VPKPCCVPTELSA				
	80	90	100		
	ISMLYLDENEKVVLKNYQEMVVEGCGCR				

41. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
COP4	LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD				
		50	60	70	
	HFNSTN--H-AVVQTLVNNMNPCK-VPKPCCVPTELSA				
	80	90	100		
	ISMLYLDENEKVVLKNYQEMVVEGCGCR				

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42. The protein of claim 21 or 22 comprising the amino acid sequences:

```
1      10      20      30      40
COP5   LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPPFLAD
              50      60      70
        HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80      90     100
        ISMLYLDENEKVVVLKNYQEMVVEGCGCR
```

43. The protein of claim 21 or 22 comprising the amino acid sequences:

```
1      10      20      30      40
COP7   LYVDFS-DVGWDDWIVAPPGYHAFYCHGECPPFLAD
              50      60      70
        HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80      90     100
        ISMLYLDENEKVVVLKNYQEMVVEGCGCR
```

44. The protein of claim 21 or 22 comprising the amino acid sequences:

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              -10
              PKHHSSRARKKNKN
1      10      20      30      40
COP16  CRRHSLYVDFS-DVGWDDWIVAPPGYQAFYCHGECPPFLAD
              50      60      70
        HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80      90     100
        ISMLYLDENEKVVVLKNYQEMVVEGCGCR
```

45. The protein of claim 21 or 22 comprising the product of expression of a DNA in a procaryotic cell.

46. A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

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47. The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A.

48. The DNA sequence of claim 46 encoding:

1 10 20 30 40  
OP1 LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

49. The DNA sequence of claim 46 encoding:

1 10 20 30 40  
OP1 CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

50. A cell line engineered to express the protein of claim 21 or 22.

51. The protein of claim 21 having a half maximum bone forming activity of about 20 - 25 ng per 25 mg of implant.

52. A biocompatible, in vivo biodegradable deglycosylated collagenous matrix defining pores of dimensions sufficient to permit influx, proliferation, and differentiation of migratory progenitor cells from the body of a mammal.

53. The matrix of claim 52 comprising close-packed particulate matter having a particle size within the range of 70-850 nm.

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54. The matrix of claim 53 wherein said particulate matter has a particle size within the range of 70-420 mm.

55. The matrix of claim 52 defining a shape to span a non-union fracture in said mammal.

56. The matrix of claim 52 comprising demineralized, protein-extracted, deglycosylated, particulate xenogenic bone.

57. The matrix of claim 52 comprising a material selected from the group consisting of hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted, deglycosylated xenogenic bone, and mixtures thereof.

58. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

substantially pure osteogenic protein capable of inducing endochondral bone formation in said mammal disposed in said matrix and accessible to said cells.

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59. The device of claim 1, 2, or 58 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70-850 mm.

60. The device of claim 1, 2, or 58 wherein said particulate matter has a particle size within the range of 70-420 mm.

61. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein-extracted, particulate, allogenic bone.

62. The device of claim 1, 2, or 58 wherein said matrix comprises a material selected from the group consisting of collagen, hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted allogenic bone, and mixtures thereof.

63. The device of claim 1, 2, or 58 wherein said matrix is shaped to span a non-union fracture in said mammal.

64. The device of claim 1, 2, or 58 disposed within the marrow cavity of allogenic bone.

65. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein extracted, particulate, deglycosylated xenogeneic bone.

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66. The device of claim 65 wherein said matrix is treated with a protease.

67. The device of claim 58 wherein said osteogenic protein is unglycosylated.

68. The device of claim 67 wherein said osteogenic protein has an apparent molecular weight of about 27 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

69. The device of claim 58 wherein said osteogenic protein is glycosylated.

70. The device of claim 69 wherein said osteogenic protein has an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

71. The device of claim 58 wherein said osteogenic protein comprises a pair of polypeptide chains.

72. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 14 kD and the other has an apparent molecular weight of about 16 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

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73. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 16 kD and the other has an apparent molecular weight of about 18 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

74. The device of claim 58 wherein said osteogenic protein has the approximate amino acid composition set forth below:

<u>Amino acid residue</u>	<u>Rel. no. res./molec.</u>	<u>Amino acid residue</u>	<u>Rel. no. res./molec.</u>
Aspartic acid/	22	Tyrosine	11
Asparagine		Valine	14
Glutamic acid/	24	Methionine	3
Glutamine		Cysteine	16
Serine	24	Isoleucine	15
Glycine	29	Leucine	15
Histidine	5	Proline	14
Arginine	13	Phenylalanine	7
Threonine	11	Tryptophan	ND
Alanine	18		
Lysine	12		

75. The device of claim 58 wherein said osteogenic protein comprises the amino acid sequence:

VPKPCCAPT



76. The device of claim 1 or 58 wherein the half maximum bone inducing activity of said protein is 0.8 to 1.0 ng per mg of said matrix.

77. A method of inducing local cartilage or bone formation in a mammal comprising the step of implanting the device of claim 1, 2, or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

78. A method of inducing endochondral bone formation in a mammal comprising the step of implanting the device of claim 1 or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

79. A method of inducing endochondral bone formation in a non-union fracture in a mammal comprising the step of implanting in the fracture in said mammal the device of claim 63.

80. Antibodies reactive with an epitope of the protein of claim 21 or 22.

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B<sup>8</sup>

add  
H<sup>2</sup>

add  
J<sup>1</sup>

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